have the composition of compound V, but did not melt at 330°.

Anal. Calcd. for $C_7H_9N_3O_4$: C, 42.21; H, 4.56; N, 21.10. Found: C, 41.68; H, 5.00; N, 20.96.

Diethyl acetal of 2,4-dimethoxy-s-triazinyl-6-ketene (VI). Diethyl acetal of 2,4-dichloro-*s*-triazinyl-6-ketene (III, 2.64 g., 0.01 mole) was added to the solution of sodium (0.46 g., 0.02 mole) in 25 ml. of methanol. The reaction mixture was refluxed for 5 min. After standing at room temperature overnight, the sodium chloride formed was filtered off and the methanol removed *in vacuo* to give a yellow colored oil. Treatment of the oil with hot benzene resulted in the precipitation of a further small amount of sodium chloride which was removed by filtration. Upon cooling of the filtrate to room temperature, it formed a very stiff gel. After standing for 2 days, the gel was subjected to a vacuum of 3 mm. The gel did not break upon this procedure, but had to be heated to remove the solvent which occurred between 60–75° (3 mm.). Attempted distillation of the remaining oil was unsuccessful. Distillation did not occur applying a vacuum of 0.7 mm. and heating the bath to 160°. At this temperature, the content of the distillation flask solidified. The solid formed was treated with 15 ml. of cold petroleum ether (b.p. 90–97°) to give a yellow powder which was filtered. The powder was extracted with 15 ml. of hot petroleum ether (b.p. 90–97°). From this extract yellow needles precipitated which, after two further recrystallizations from the petroleum ether, melted at 78–80° and were found to be compound VI.

Anal. Calcd. for $C_{11}H_{17}N_3O_4$: C, 51.75; H, 6.71; N, 16.47. Found: C, 51.61; H, 6.76; N, 17.20.

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Production of Pyrophosphate from S-*n*-Butylphosphorothioate[1,2]

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Esters of thiols (such as coenzyme A) and phosphoric acid have been suggested as intermediates in biological phosphate transfers[3]; and *S*-*n*-butylphosphorothioate accomplishes the nonenzymic phosphorylation of alcohols and even acetic acid,[4] the latter yielding acetyl phosphate.

In view of these findings, it was of interest to determine if *S*-*n*-butylphosphorothioate could transfer phosphate to phosphoric acid to yield pyrophosphate.

We wish to report that pyrophosphate is produced when disodium *S*-*n*-butylphosphorothioate is treated with phosphoric acid, either in the presence or absence of iodine. The best yields of pyrophosphate (determined roughly by comparisons of paper chromatograms of known amounts of pyrophosphate with paper chromatograms of the reaction products) appeared to be obtained when the reaction was run in dioxane in the presence of iodine. Wieland and Lambert have shown that iodine had a beneficial effect on the phosphorylation of alcohols.[4] As iodine reacts immediately and quantitatively with *S*-*n*-butylphosphorothioate, the increased yields in the presence of iodine may be caused partly by facilitation of the dehydration of phosphoric acid by the hydriodic acid produced.

The pyrophosphate may be formed either by a direct phosphorylation of phosphoric acid or by the reaction of two moles of ester to give a thiopyrophosphate ester which is hydrolyzed to the products.

$$\begin{tikzcd} n\text{-}C_4H_9SPO_3H_2 + H_3PO_4 \arrow[r] \arrow[d] & H_4P_2O_7 + n\text{-}C_4H_9SH \\ n\text{-}C_4H_9SP_2O_6H_3 \arrow[ur] & \end{tikzcd}$$

Pyrophosphate was reported as an impurity in the preparation of *S*-*n*-propylphosphorothioate.[3a] It was not clear whether the pyrophosphate was formed during the reaction of the thiol with phosphorous oxychloride or afterwards from the thioester itself.[5] It is known that phosphorus oxychloride and phosphoric acid yield pyrophosphoric acid.[6]

Occasionally, paper chromatography of acidified aqueous solutions of barium or disodium *S*-*n*-butylphosphorothioate showed evidence of pyrophosphate. Because the salts initially were pure, it is probable that pyrophosphate formation proceeded as indicated in the equations above.

Precise quantitative data on yields are lacking, but visual estimates of the yields from a comparison of the size and intensity of the spots of known and unknown quantities of pyrophosphate were between 40 and 70%.

EXPERIMENTAL

Barium and disodium S-n-butylphosphorothioate. The barium salt (as its alcoholate) was prepared by the method of Wieland and Lambert[4] with modifications.[7] The sodium salt was prepared by trituration of the barium salt with sodium sulfate.[7]

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(1) Taken from the B.S. thesis of Virginia Opshelor, University of Pennsylvania, 1958.

(2) We gratefully acknowledge the support of the National Institutes of Health (Grant A-1023) for part of this work.

(3)(a) E. O'F. Walsh, *Nature*, **169**, 546 (1952); (b) G. Feuer and M. Wollemann, *Acta Physiol. Acad. Sci. Hung.*, **7**, 343 (1955); *Chem. Abstr.*, **50**, 1097 (1956); M. Wollemann, *Acta Physiol. Acad. Sci. Hung.*, **10**, 171 (1956); *Chem. Abstr.*, **51**, 6730 (1957); T. Stadtman, P. Elliott, and L. Tiemann, *J. Biol. Chem.*, **231**, 961 (1958).

(4) T. Wieland and R. Lambert, *Chem. Ber.*, **89**, 2476 (1956).

(5) In a private communication, Dr. E. O'F. Walsh was of the opinion that the pyrophosphate impurity was formed in a reaction of phosphoric acid with *S*-*n*-propylphosphorothioate.

(6) A. Geuther, *J. prakt. Chem.*, **116**, 359 (1873).

(7) O. B. Ramsay, Ph.D. thesis, University of Pennsylvania, February 1960.

(8) Analysis by Galbraith Laboratories, Knoxville, Tenn.

Anal.[7,8] Calcd. for $C_4H_9O_3PSNa_2$: C, 22.43; H, 4.24; S, 14.97. Found: C, 22.17; H, 4.56; S, 14.76.

An average of five iodometric determinations indicated that the disodium salt was 99.1% pure.

Separation of ortho- and pyrophosphate by paper chromatography. The solvent system used was methanol (80 vol.), 90% formic acid (15 vol.), and water (5 vol.).[9] Development by descending chromatography on Whatman No. 1 filter paper in a cylindrical, covered glass jar took approximately 5 hr. at room temperature. The chromatograms were dried and sprayed with a molybdate reagent.[10] Yellow spots corresponding to the orthophosphate appeared immediately; after the chromatograms were dried overnight, faint spots produced by the hydrolyzed pyrophosphate were also visible.

The chromatograms were exposed to ultraviolet light at a distance of about 10 cm. for 30 min. All the blue color except that produced by the phosphomolybdenum complex was removed by treatment with ammonia vapors.[9]

The following R_f values were observed: orthophosphate, 0.70; pyrophosphate, 0.55. The R_f values varied somewhat depending on concentration. Some streaking was observed with pyrophosphate. Washing the filter paper with an alcoholic solution of 8-hydroxyquinoline[10,11] prior to running the chromatogram did not improve the results.

Paper chromatography of S-n-butylphosphorothioate. When acidified (sulfuric acid) aqueous solutions of pure barium or disodium *S*-*n*-butylphosphorothioate were chromatographed by the method described in the preceding section, considerable streaking was obtained, as well as a spot corresponding to orthophosphate. The streaked portion of the chromatogram was a bright blue as contrasted with the more violet orthophosphate spots. On several chromatograms, but not on all, a spot was obtained corresponding to pyrophosphate.

Treatment of these chromatograms with a mercuric chloride, nitro-prusside and potassium cyanide reagent[4] produced crimson spots, R_f = .85. These may be due to unhydrolyzed ester. On some chromatograms treated with the phosphate-detecting reagents, a faint blue spot appeared at the same R_f value.

Phosphorylation of orthophosphate. Disodium *S*-*n*-butylphosphorothioate (17.3 mg., 0.0809 mmole) and disodium orthophosphate ($Na_2HPO_4 \cdot 7H_2O$, 44.3 mg., 0.163 mmole) were dissolved in 0.21 ml. of 20% sulfuric acid and allowed to stand at room temperature for 24 hr. A paper chromatogram of the resulting solution, diluted to 2 ml., showed spots (R_f = 0.41 and 0.70) corresponding to those (R_f = 0.41 and 0.71) produced by a standard of 30.7 mg. (0.0688 mmole) of sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) and 42.1 mg. of disodium orthophosphate in 0.21 ml. of 20% sulfuric acid, diluted to 2 ml. The reaction mixture and the standard mixture were chromatographed on the same sheet of filter paper. Inspection of the chromatogram indicated that the amount of pyrophosphate produced in the reaction was roughly one-third to one-half the amount in the standard, and that the amount of orthophosphate was about one-third less than that in the standard. Other runs under similar conditions gave substantially the same results.

"Oxidative" phosphorylation of orthophosphate. Disodium *S*-*n*-butylphosphorothioate (80.4 mg., 0.375 mmole) was mixed with disodium orthophosphate ($Na_2HPO_4 \cdot 7H_2O$, 184.9 mg., 0.690 mmole), 20% sulfuric acid (0.08 ml.), and an aqueous solution of iodine (0.026 ml.). The amount of iodine solution used varied from run to run, but only enough iodine was added until the color of free iodine persisted. The reaction mixture was heated for 15 min. in a bath of boiling water. A paper chromatogram showed spots corresponding to orthophosphate and pyrophosphate. The amount of pyrophosphate produced appeared to be intermediate between standards of 50.9 mg. (0.114 mmole) and 113.1 mg. (0.253 mmole) of tetrasodium pyrophosphate pentahydrate. Therefore, the yield of pyrophosphate was between 30 and 67%.

Disodium *S*-*n*-butylphosphorothioate (9.7 mg., 0.0453 mmole) was mixed with 85% phosphoric acid (0.005 ml.) in dioxane (0.5 ml.) and heated in a bath of boiling water for 15 min. during which 0.4 ml. of a dioxane solution of iodine was added before the iodine color persisted. The solution was allowed to evaporate overnight and the residue was diluted with 1 ml. of water. Spots for both orthophosphate and pyrophosphate were obtained on a paper chromatogram. There was some streaking. The amount of pyrophosphate produced was nearly as large as that in a pyrophosphate standard (14.2 mg. of $Na_4P_2O_7 \cdot 10H_2O$, 0.0318 m-mole) which was subjected to the same treatment as the disodium *S*-*n*-butylthiophosphate. The standard was run on the same chromatogram. The yield would be of the order of 70%.

A sample of orthophosphoric acid (0.005 ml. in 0.5 ml. of dioxane and 0.05 ml. of iodine solution) showed no streaking and its R_f value was unchanged.

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p-Methylthiobenzylamine

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In the course of some other work, *p*-methylthiobenzylamine was needed. The corresponding *ortho* compound had been prepared from thiosalicyclic acid by methylation, conversion to the amide and reduction with lithium aluminum hydride,[1] but a more direct synthesis was desired.

The ease with which a suitably activated fluorine attached to an aromatic nucleus is displaced by nucleophilic agents,[2] the marked nucleophilicity of the RS^- anion,[2] and the commercial availability of *p*-fluorobenzonitrile suggested a simpler method of synthesizing *p*-methylthiobenzonitrile than the use of the Sandmeyer reaction with *p*-methylthioaniline.[3] Reduction of the nitrile with lithium aluminum hydride should yield the benzylamine.

The expectations were realized. The displacement reaction of sodium methylmercaptide with *p*-fluorobenzonitrile afforded 82% yield of crude *p*-methylthiobenzonitrile, which on reduction gave 72% yield of *p*-methylthiobenzylamine.

The *p*-methylthiobenzonitrile was identified by its melting point, analysis, alkaline hydrolysis to

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